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Letter to the Editor

The need for proper voucherizing in phylogenetic studies of birds**1. Introduction**

A recent paper published in *Molecular Phylogenetics and Evolution* raises an important issue in systematic biology, especially in avian systematics. Increasing numbers of avian molecular systematic studies rely on unvouchered genetic material for part or all of their sampling, a trend that we believe needs to be addressed by the systematic community. In the study that brought this issue to the forefront, [Olsson et al. \(2006\)](#) treated a difficult-to-identify genus of birds (*Cettia*) across a diverse region of South and Southeast Asia, with sampling based entirely on blood or feathers taken from 13 taxa at nine localities in China, India, Indonesia, Japan, Malaysia, Myanmar, Spain, and Vietnam. This approach is—at first glance, at least—attractive, because birds can be sampled quickly and efficiently, without the need to sacrifice the individual. Upon more careful consideration, however, sampling for phylogenetic and phylogeographic studies without full and appropriate voucher specimens deposited in publicly accessible scientific collections is both irresponsible and unconvincing, for reasons discussed below. Put simply, because phenotypic information is unavailable, we do not believe that such unvouchered studies fit the definition of “science.”

1.1. Reliance on field identifications

Field situations are not always the best conditions under which to make difficult identifications. In our experience, a small but non-trivial proportion of specimens collected on expeditions is misidentified in the field, even by skilled field ornithologists; these errors are rectified only when vouchers are compared to museum reference collections. In particular, Old World warblers (“Sylviidae”) are infamous for their complexity and identification challenges ([Grimmet et al., 1999](#); [Robson, 2000](#); [Baker, 1997](#)), so sampling for studies of such difficult groups should rely on a high percentage, preferably 100%, of the individuals vouchered. [Olsson et al. \(2006\)](#), however, based sweeping conclusions—including reallocation of key populations to different species—based solely on field identifications and single samples per population. Because the individual birds

sampled are no longer accessible, their study cannot be replicated (i.e., identifications based on phenotype cannot be rechecked), and therefore violates basic tenets of the scientific method.

1.2. Systematics uses multiple character sets

Systematics is by nature a science that relies on multiple and diverse character suites ([Wiley, 1981](#))—species-level systematics commonly refers to evidence from plumage coloration, vocalizations, size and shape, and molecular characters gathered from series of individuals. Indeed, the long history of systematics has shown the perils of reliance on single character suites (e.g., [Cracraft, 1982](#)), a point echoed more quantitatively in molecular systematics with the realization that single gene trees may not represent population trees ([Brower et al., 1996](#)). The point, however, is more general: conclusions regarding phylogeny, relationships, and especially species limits require multiple character sets—the ability for cross-referencing among characters is critical if conclusions are to be at all robust.

The voucher-free approach followed by [Olsson et al. \(2006\)](#) falls into this perilous zone. For instance, among their more striking results is the polyphyly of samples attributed to *Cettia flavolivacea*, with samples from China and Vietnam grouping with *C. vulcania*—certainly, the interpretation they offer is a possibility, but other possibilities could be considered were proper vouchers prepared. First, the field identification could be correct, and certain populations of *C. flavolivacea* are in fact more closely related to *C. vulcania* than to other populations of *C. flavolivacea*: result—taxonomy must be changed. Second, the field identification could be incorrect; the individuals sampled are in fact attributable to *C. vulcania* but demonstrate a new distributional record: result—the known distribution of *C. vulcania* is revised. Third, the field identification could be incorrect and the samples represent a previously undescribed taxon: result—a new taxon needs description. These possibilities can be tested only via comparative study of the vouchers for the molecular samples with the type series of *C. vulcania* and *C. flavolivacea*, which is not possible in the present case: distinguishing between these scenarios in the present case must await a new study with vouchered samples. Although true that additional molecular characters can likely be extracted from the blood and feather samples, the systematics community is not able to access

information from more diverse character suites: plumage variation, morphometrics, and vocalizations are—put simply—not available for analysis. Molecular phylogenies with such limitations are problematic and cannot be verified, leaving open questions of accurate reconstruction of relationships of these organisms.

1.3. Contribution to the scientific community

The systematic community, and the museum community in particular, has thrived on a tradition of open exchange of material among researchers and institutions. It is hard to imagine other fields giving hard-earned data so freely to other researchers, because that is really what specimens are, in the hands of trained systematists. This system thrives when all or most of the participants not only use specimen material, but also expend the time and effort to collect *new* material. Although probably better than collecting no material at all, collecting unvouchered genetic samples provides little to the systematic community, and in fact sets a bad example in developing countries regarding the proper way to conduct research in phylogenetics and phylogeography. Numerous commentaries have emphasized the enormous positive contributions that specimen material makes to a broader scientific understanding of biodiversity (Peterson and Lanyon, 1992; Remsen, 1995; Winker, 1996; Winker et al., 1996; Edwards et al., 2005).

2. Conclusions

This letter is intended to help to improve the quality of systematic research being published by colleagues from around the world. A key feature of the scientific method is the emphasis on the potential for replication of the study and its results. Analyses based on voucher-less samples cannot be replicated because the identification and phenotypic characteristics of the individual from which the sample was obtained cannot be corroborated. Further, this type of analysis will frequently lead to erroneous results, as well as to an overly simplistic view of systematics, in which conclusions are based on too few character sets and on too narrow a base of information. Carefully prepared and preserved voucher specimens can offer vast and unanticipated additional information—for instance, migratory status can often be established based on feather shape, migratory origin can be extracted from stable isotope profiles, and misidentifications can be rectified based on plumage and morphometrics.

Of course, we understand the occasional need for representation of key taxa based on fragmentary specimen material when no other means exists for their inclusion, especially when dealing with endangered species, politically sensitive or dangerous regions, or marked individuals in ongoing population studies. Still, we see many papers based on sampling that is entirely, mostly, or partially voucher-free (Roy, 1997; Mann et al., 2006; Olsson et al., 2005, 2006), raising doubts that vouchering was really not feasible. Other studies (e.g. Cibois, 2003; Ericson et al., 2006;

Qu et al., 2006) do not distinguish between vouchered and unvouchered material: muscle tissue, blood, and feathers are listed as sources in the methods, but the sample table, if one exists, does not distinguish among the sources.

We recommend the following minimum standards in presentation of sources of molecular data: (1) repository institution name and catalog number for the voucher for each sample, (2) geographic locality from whence the sample was taken, and (3) nature of the voucher (e.g., study skin, skeleton, photograph, blood only). Although some of the concerns discussed above were addressed for mammals by Ruedas et al. (2000) and for African birds by Bates et al. (2004), the community of authors and reviewers does not appear to have noticed—or fully taken to heart—these messages. We urge our colleagues to consider carefully the implications of developing voucher-free systematic studies, and we urge editors and reviewers of molecular systematic studies to weigh these points in decisions regarding the merit of manuscripts under consideration for publication.

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